

WHAT IS CLAIMED IS:

1 1. An isolated infectious chimeric parainfluenza virus (PIV) comprising a
2 major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein
3 (L), and a human PIV (HPIV) vector genome or antigenome that is modified to encode a
4 chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments,
5 or epitopes of a second, antigenically distinct HPIV.

1 2. The chimeric PIV of claim 1, wherein one or more heterologous genome
2 segment(s) of the second, antigenically distinct HPIV encoding said one or more antigenic
3 domains, fragments, or epitopes is/are substituted within the HPIV vector genome or
4 antigenome to encode said chimeric glycoprotein.

1 3. The chimeric PIV of claim 2, wherein said one or more heterologous
2 genome segment(s) encode(s) one or more glycoprotein ectodomain(s) substituted for one or
3 more corresponding glycoprotein ectodomain(s) in the vector genome or antigenome.

1 4. The chimeric PIV of claim 2, wherein heterologous genome segments
2 encoding both a glycoprotein ectodomain and transmembrane region are substituted for
3 counterpart glycoprotein ecto- and transmembrane domains in the vector genome or
4 antigenome.

1 5. The chimeric PIV of claim 1, wherein said chimeric glycoprotein is
2 selected from HPIV HN or F glycoproteins.

1 6. The chimeric PIV of claim 1, wherein the (HPIV) vector genome or
2 antigenome is modified to encode multiple chimeric glycoproteins.

1 7. The chimeric PIV of claim 1, wherein the HPIV vector genome or
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct
3 HPIV is selected from HPIV1 or HPIV2.

1 8. The chimeric PIV of claim 7, wherein the HPIV vector genome or
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct
3 HPIV is HPIV2.

1 9. The chimeric PIV of claim 8, wherein one or more glycoprotein
2 ectodomain(s) of HPIV2 is/are substituted for one or more corresponding glycoprotein
3 ectodomain(s) in the HPIV3 vector genome or antigenome.

1 10. The chimeric PIV of claim 9, wherein both glycoprotein ectodomain(s)
2 of HPIV2 HN and F glycoproteins are substituted for corresponding HN and F glycoprotein
3 ectodomains in the HPIV3 vector genome or antigenome.

1 11. The chimeric PIV of claim 10, which is rPIV3-2TM.

1 12. The chimeric PIV of claim 10, which is further modified to incorporate
2 one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*.
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1 13. The chimeric PIV of claim 12, which is rPIV3-2TM_{*cp45*}

1 14. The chimeric PIV of claim 8, wherein PIV2 ectodomain and
2 transmembrane regions of one or both HN and/or F glycoproteins is/are fused to one or more
3 corresponding PIV3 cytoplasmic tail region(s).

1 15. The chimeric PIV of claim 14, wherein ectodomain and transmembrane
2 regions of both PIV2 HN and F glycoproteins are fused to corresponding PIV3 HN and F
3 cytoplasmic tail regions.

1 16. The chimeric PIV of claim 15, which is rPIV3-2CT.

1 17. The chimeric PIV of claim 16, which is further modified to incorporate
2 one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*.
3

1 18. The chimeric PIV of claim 15, which is rPIV3-2CT_{*cp45*}.

1 19. The chimeric PIV of claim 1, which is further modified to incorporate
2 one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*
3 selected from mutations specifying an amino acid substitution in the L protein at a position
4 corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a position
5 corresponding to residues Val96 or Ser389 of JS *cp45*, in the C protein at a position
6 corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the
7 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a
8 mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS *cp45*

1 20. The chimeric PIV of claim 1, wherein a plurality of heterologous genes
2 or genome segments encoding antigenic determinants of multiple heterologous PIVs are added
3 to or incorporated within the partial or complete HPIV vector genome or antigenome.

1 21. The chimeric PIV of claim 20, wherein said plurality of heterologous
2 genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 and
3 are added to or incorporated within a partial or complete HPIV3 vector genome or antigenome.

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1 22. The chimeric PIV of claim 20, wherein the chimeric genome or
2 antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or
3 epitopes from two or more different HPIVs.

1 23. The chimeric PIV of claim 1, wherein the chimeric PIV genome or
2 antigenome is attenuated by addition or incorporation of one gene or cis-acting regulatory
3 element from a bovine PIV3 (BPIV3).

1 24. The chimeric PIV of claim 1, wherein the chimeric PIV genome or
2 antigenome incorporates one or more heterologous, non-coding non-sense polynucleotide
3 sequence(s).

1 25. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or
3 epitopes from both HPIV3 JS and HPIV1 or HPIV2.

1 26. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome is modified by introduction of an attenuating mutation involving an amino
3 acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

1 27. The chimeric PIV of claim 26, wherein phenylalanine at position
2 456 of the HPIV3 L protein is substituted by leucine.

1 28. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome incorporates one or more heterologous gene(s) or genome segment(s)
3 encoding one or more respiratory syncytial virus (RSV) F and/or G glycoprotein(s) or
4 immunogenic domain(s), fragment(s), or epitope(s) thereof.

1 29. The chimeric PIV of claim 1 which is a virus.

1 30. The chimeric PIV of claim 1 which is a subviral particle.

1 31. A method for stimulating the immune system of an individual to induce
2 protection against PIV which comprises administering to the individual an immunologically
3 sufficient amount of the chimeric PIV of claim 1 combined with a physiologically acceptable
4 carrier.

1 32. The method of claim 31, wherein the chimeric PIV is administered in a
2 dose of 10^3 to 10^7 PFU.

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2 33. The method of claim 31, wherein the chimeric PIV is administered to
the upper respiratory tract.

1 34. The method of claim 31, wherein the chimeric PIV is administered by
2 spray, droplet or aerosol.

1 35. The method of claim 31, wherein the vector genome or antigenome is of
2 human PIV3 (HPIV3) and the chimeric PIV elicits an immune response against HPIV1 and/or
3 HPIV2.

1 36. The method of claim 31, wherein the chimeric PIV elicits a polyspecific
2 immune response against multiple human PIVs.

1 37. The method of claim 31, wherein a first, chimeric PIV and a second PIV
2 are administered sequentially or simultaneously to elicit a polyspecific immune response.

1 38. The method of claim 37, wherein the second PIV is a second, chimeric .
2 PIV according to claim 1.

1 39. The method of claim 37, wherein the first, chimeric PIV and second PIV
2 are administered simultaneously in a mixture.

1 40. The method of claim 37, wherein the first and second chimeric PIVs are
2 bear the same or different heterologous antigenic determinant(s).

1 41. The method of claim 37, wherein the first chimeric PIV elicits an
2 immune response against HPIV3 and the second chimeric PIV elicits an immune response
3 against HPIV1 or HPIV2.

1 42. The method of claim 37, wherein the second chimeric PIV incorporates
2 one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic
3 determinant(s) of respiratory syncytial virus (RSV).

1 43. The method of claim 42, wherein both the first and second chimeric
2 PIVs elicit an immune response against RSV.

1 44. The method of claim 43, wherein the first chimeric PIV is administered
2 initially in a vaccination protocol and the second chimeric PIV is administered subsequently in
3 the vaccination protocol to provide initial immunization against HPIV3 and secondary
4 immunization against HPIV1 or HPIV2 and to provide initial and secondary, booster
5 immunization against RSV.

1 45. The method of claim 37, wherein the first, chimeric PIV incorporates at
2 least one and up to a full complement of attenuating mutations present within PIV3 JS cp45
3 selected from mutations specifying an amino acid substitution in the L protein at a position
4 corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a position
5 corresponding to residues Val96 or Ser389 of JS cp45, in the C protein at a position
6 corresponding to Ile96 of JS cp45, a nucleotide substitution in a 3' leader sequence of the
7 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a
8 mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS cp45.

1 46. An immunogenic composition to elicit an immune response against PIV
2 comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in a
3 physiologically acceptable carrier.

1 47. The immunogenic composition of claim 46, formulated in a dose of 10^3
2 to 10^7 PFU.

1 48. The immunogenic composition of claim 46, formulated for
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1 49. The immunogenic composition of claim 46, wherein the chimeric PIV
2 elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2 and
3 HPIV3.

1 50. The immunogenic composition of claim 46, wherein the chimeric PIV
2 elicits an immune response against HPIV3 and another virus selected from HPIV1, HPIV2,
3 and respiratory syncytial virus (RSV).

1 51. The immunogenic composition of claim 46, further comprising a
2 second, chimeric PIV according to claim 1.

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1 52. The immunogenic composition of claim 51, wherein the first chimeric
2 PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits an immune
3 response against HPIV1 or HPIV2, and wherein both the first and second chimeric PIVs elicit
4 an immune response against RSV.

1 53. An isolated polynucleotide comprising a chimeric PIV genome or
2 antigenome which includes a human PIV (HPIV) vector genome or antigenome modified to
3 encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains,
4 fragments, or epitopes of a second, antigenically distinct HPIV.

1 54. The isolated polynucleotide of claim 53, wherein one or more
2 heterologous genome segment(s) encoding the antigenic domains, fragments, or epitopes of
3 said second, antigenically distinct HPIV is/are substituted for one or more counterpart genome
4 segment(s) in the HPIV vector genome or antigenome.

1 55. The isolated polynucleotide of claim 53, wherein, the chimeric genome
2 or antigenome incorporates at least one and up to a full complement of attenuating mutations
3 present within PIV3 JS *cp45*.

1 56. A method for producing an infectious attenuated chimeric PIV particle
2 from one or more isolated polynucleotide molecules encoding said PIV, comprising:

3 expressing in a cell or cell-free lysate an expression vector comprising an
4 isolated polynucleotide comprising a vector genome or antigenome modified to encode a
5 chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments,
6 or epitopes of a second, antigenically distinct HPIV, and PIV N, P, and L proteins.

1 57. The method of claim 56, wherein the chimeric PIV genome or
2 antigenome and the N, P, and L proteins are expressed by two or more different expression
3 vectors.

1 58. An expression vector comprising an operably linked transcriptional
2 promoter, a polynucleotide sequence which includes a vector genome or antigenome modified
3 to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains,
4 fragments, or epitopes of a second, antigenically distinct HPIV, and a transcriptional
5 terminator.